

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Biochemical Studies on "Sotetsu"

(*Cycas revoluta* Thunb.) Part V.

On the Chemical Constituents of "Sotetsu" pollen. I.

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(Received September 25, 1935.)

On the chemical constituent of several pollens, from the biochemical or pharmaceutical point of view, it has been studied by many investigators; but there is no chemical investigation as to the sotetsu pollen. The writer, therefore, has taken it on himself to investigate the biochemistry of sotetsu pollen.

From the biological point of view, the plant pollen corresponding to reproductive cell of the animal sperm, therefore arises question as to the presence of substances, which were contained in the sperm, yet had not been discovered in the vegetable kingdom. Especially in the case of sotetsu pollen, from which develops the sperm, above question is deeper than in the other cases.

That is to say, the substances in question are spermine in organic bases and protamin in proteins. The writer, therefore, to give a solution to the question experimented on the sotetsu pollen.

By some authors hitherto, the organic bases isolated from the several pollens, are as following table:

Investigator.	Pollen source.	Organic bases isolated.
Heyl ⁽¹⁾ , ⁽²⁾	Ragweed	Adenine, Histidine, Arginine, Lysine, Agmatine, Guanosine, Betaine.
Anderson & Kulp ⁽³⁾	Corn.	Choline, <i>l</i> -proline.
Kiesel ⁽⁴⁾	<i>Pinus silvestris</i> .	Adenine, Arginine, Choline, Histidine, Guanine.

Miyake ⁽⁵⁾	Corn.	Adenine, Choline.
Vinson ⁽⁶⁾	Corn	Adenine, Arginine, Lysine, Choline.
Kiesel & Rubin ⁽⁷⁾	Sugar beet	Adenine, Betaine, Choline.

Benjamins⁽⁸⁾ reported an extract of rye pollen reacts like histamine, when tested by the method of Dale, and regularly causes contraction of guinea-pig and cat uterus; and it suggests that the presence of histamine in this pollen, and not in others investigated, indicates the special significance of rye pollen in the physiology of hay fever.

EXPERIMENTAL PART

The pollen used in this experiment are produced in Okinawa island 1935 and collected on June 20~25 in that year and immediately dried at 50°C, stored carefully in a sulphuric acid-desiccator.

I. General Composition of Sotetsu pollen.

The general chemical composition of sotetsu pollen is as follows:

	Sample A (Produced near Naha City.)		Sample B (Produced at Hanedi Village)	
	In 100 parts of original sample	In 100 parts of dry matter	In 100 parts of original sample	In 100 parts of dry matter
Moisture	21.87	—	14.24	—
Dry matter	78.13	100.00	85.76	100.00
Crude protein	25.74	32.94	29.00	33.81
Protein	19.09	24.44	23.50	27.38
Alcohol extract	15.29	19.57	15.80	18.42
Starch*	0	0	0	0
Dextrin	1.55	1.98	—	—
Reducing sugar (as glucose) }	0.33	0.42	0.32	0.37
Non reducing sugar (as sucrose) }	1.02	1.31	1.31	1.53
Crude fibre	24.32	31.13	26.22	30.57
Crude ash	4.54	5.81	4.77	5.56
Total N	4.12	5.27	4.64	5.41
Protein N	3.05	3.91	3.76	4.38
Non protein N	1.07	1.36	0.88	1.03
Organic base N	0.12	0.15	—	—
Ammonia N	0.04	0.05	—	—

* The sample gives no iodine test for starch.

II. *Is there Formaldehyde-containing substance in Sotetsu pollen?*

The writer⁹ reported that the sotetsu seed contains fairly amount of formaldehyde-containing substance, and since it is hydrolyzed by emulsin it is probably a β -glucoside. Also on the sotetsu pollen, the reaction of formaldehyde—Rimini's, Vitali's and Yoder-Taggart's method—was experimented; but every reaction resulted the negative, in both cases with and without addition of H_2SO_4 in steam distillation.

III. *Isolation and Identification of Organic Bases.*

For the isolation of organic bases and protamine, 1 kg of the sotetsu pollen was extracted with ether and then with 85% alcohol at below 50°C in several times. The alcohol extract was evaporated under reduced pressure to a small volume for expelling the alcohol; then fat and lipoid were removed by ether, and several impurities by Pb-acetate. The excess of Pb^{++} was removed by H_2S and the mother liquor from the Pb-sulphide was evaporated to a small volume. The concentrated solution thus obtained was diluted with 5% H_2SO_4 , and then researches were carried out on the organic bases by adding the phosphotungstic acid to above solution. The phosphotungstic acid-precipitate was decomposed by $\text{Ba}(\text{OH})_2$, and excess of Ba^{++} removed by CO_2 and the solution fractionated into five fractions.

(A) Volatile base (Ammonia).

The diluted solution of free bases obtained by above treatment was distilled under reduced pressure, and the distillate was received into dilute HCl. The hydrochloride of volatile base was in soluble in alcohol, and gave intensive Nessler's reaction.

(B) *Researches of spermine by Wrede's method.⁽¹⁰⁾

After the distillation of volatile base, the concentrated residual solution was exactly neutralized with H_3PO_4 and after addition of 96% alcohol, the solution was allowed to stand for one night in ice box, but the characteristic phosphate of spermine was not formed at all.

(C) Precipitate by AgNO_3 (Adenine).

To remove the PO_4''' in the solution obtained from above experiment, the $\text{Ba}(\text{OH})_2$ was added to it, and excess of Ba^{++} removed by CO_2 and the solution of free base was slightly acidified with HNO_3 , and the precipitate prepared by AgNO_3 . From the AgNO_3 -precipitate, by decomposing with HCl, the hydrochloride of the base (0.15 g) was crystallized out. Its chloroaurate forms yellow prisms and melts at 262°C with decomposition.

Anal.: Subst.=0.1107 g; Au=0.0524 g

Found: Au=47.34%

Calc. for $(C_5H_5N_3 \cdot 2HCl \cdot 2AuCl_3 \cdot H_2O)$: Au=47.35%

The picrate of characteristic hairy needles is greenish yellow and sparingly soluble in water and it decomposes at 282°C.

(D) Precipitate by $AgNO_3$ and $Ba(OH)_2$ (Adenine).

The precipitate, prepared by $AgNO_3$ and $Ba(OH)_2$, was treated with HCl and H_2SO_4 to remove the Ag^+ and Ba^{++} . Then phosphotungstic acid was added to the above filtrate, and the precipitate was decomposed by $Ba(OH)_2$. The filtrate from Ba -phosphotungstate, after removing the excess of Ba^{++} by CO_2 , was concentrated to a small volume under reduced pressure; and then the solution of free base was neutralized with HNO_3 .

The nitrate freed from water was treated with cold absolute alcohol and the insoluble portion in absolute alcohol was converted into chloroaurate. Yield: 0.10 g. The chloroaurate forms yellow prisms and decomposes at 265~266°C.

Anal.: Subst.=0.0965 g; Au=0.0456 g

Found: Au=47.25%

Calc. for $(C_5H_5N_3 \cdot 2HCl \cdot 2AuCl_3 \cdot H_2O)$: Au=47.35%

The picrate crystallized in characteristic hairy needles, greenish yellow, sparingly soluble in water, decomposed at 282°C.

(E) Filtrate from $AgNO_3$ - and $Ba(OH)_2$ - Precipitate (Choline).

The filtrate was treated with HCl and H_2SO_4 to remove an excess of Ag^+ and Ba^{++} . Then phosphotungstic acid was added to the above solution, and the precipitate obtained thus was treated like the fraction (C). The solution of free base was converted into the hydrochloride by adding HCl , and the hydrochloride freed from water was treated with cold absolute alcohol.

The alcohol soluble portion gave a white precipitate by adding the saturated alcoholic solution of $HgCl_2$. To remove the Hg^{++} , the H_2S was passed into the solution from precipitate by $HgCl_2$. And when the mother liquor was evaporated to a small volume, the hygroscopic hydrochloride of the base was crystallized out. The chloroaurate formed yellow mossy crystals, and was sparingly soluble in water; the melting point was determined at 255°C.

Anal.: Subst.=0.2691 g; Au=0.1196 g

Found: Au=44.44%

Calc. for $(C_5H_{14}NOCl \cdot AuCl_3)$: Au=44.49%

The chloroplatinate formed orange yellow prisms, easily soluble in water,

decomposed at 237°C, and gave following analysis :

Anal. : Subst. = 0.3846 g ; Pt = 0.1205 g

Found : Pt = 31.33%

Calc. for $(C_5H_{14}NOCl)_2PtCl_4$: pt = 31.64%

The above results agree fairly with choline derivatives ; the yield of the base was 2.24 g as chloroaurate.

IV. *Researches of Protamine in Sotetsu pollen.*

The residue, after the alcohol extraction of 1 kg sotetsu pollen, was put into a bottle with 1% H_2SO_4 and shaken continuously for two hours, then sucked off, and the residue treated repeatedly in the same way until the H_2SO_4 extract caused no precipitate with about three times the volume of absolute alcohol.

The extracts were united, a large volume of absolute alcohol was added, and the vessel put in the room for 24 hours. Then filtered, washed with absolute alcohol, and finally with ether.

The pure white powder (24.60 g) obtained by above treatment, for the most part, consisted of inorganic matter and it was nearly negative even the biuret reaction.

SUMMARY

The principal results are summarized as follows :

(1) General constituent of sotetsu pollen was determined ; in the result it did not contained a starch.

(2) In sotetsu pollen, the formaldehyde-containing substance was not contained.

(3) The adenine and choline, in free state, were isolated from sotetsu pollen.

(4) An attempt at the isolations of spermine and protamine from sotetsu pollen was ended in negative results.

The writer wishes to express his thanks to Dr. K. Yoshimura and Prof. Dr. Y. Okuda for their having interest on the problem, and also begs to acknowledge with thanks that a part of the expense of this study was defrayed from a grant given by the Nippon Gakuzyutsu-shinkokwai.

(Sep. 22, 1935)

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- (8) C. E. Benjamins: Z. Immunitäts, **72**, 189, (1931).
- (9) K. Nishida: J. Agr. Chem. Soc. Japan, **11**, 357, (1935).
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Physiologically Active Crystalline Esters of Vitamin A.

By Sadayuki HAMANO.

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(Received for Publication September 12, 1935.)

In a previous paper⁽¹⁾ K. Kawakami and the present author⁽²⁾ have reported that they have prepared crystalline compounds of fatty acid or benzoic esters of vitamin A by condensing them with maleic anhydride, but these crystals had no physiological activity and could not be regenerated into vitamin A.

The present author has now succeeded in preparing a crystalline ester of vitamin A by treating vitamin A concentrate (Biosterin) with β -naphthoyl chloride in presence of pyridine and proved to have the activity of 7200 C. L.O. units after Carr-Price test. It readily promotes the growth of albino rats and cures the xerophthalmia. Dr. S. Kato in our institute has observed that a 1/140,000 mol methyl alcoholic solution of the crystals show absorption bands at 3595, 3385, 3060, 2920, 2816 Å besides the maximum at 3225 Å.

The crystals have the formula $C_{31}H_{36}O_2$, m p 76°, it is easily hydrolysed by alcoholic potash into β -naphthoic acid, $C_{11}H_8O_2$, and pure vitamin A, the analysis of which agrees with the formula proposed by P. Karrer, i.e., $C_{30}H_{30}O$. Thus it has been definitely proved that the crystals are the ester of β -naphthoic acid and vitamin A.

EXPERIMENTAL.

9 g of the vitamin A concentrate (6000 C.L.O.U.) prepared from the liver

oil of *theragra chalcogramma* were dissolved in 30 cc anhydrous pyridine, warmed for 30 min with a solution of β -naphthoyl chloride in 30 cc benzol in a current of CO_2 gas. After cooling, the reaction mixture was treated with 300 cc ether, washed first with water and dilute hydrochloric acid to remove pyridine, then with saturated sodium bicarbonate solution to eliminate the excess of β -naphthoic acid, and washed again with water. The ethereal solution gave on evaporation a viscous residue which when dissolved in acetone and cooled at $-40 \sim -80^\circ$ formed a yellow crystalline precipitate which was insoluble in petroleum ether, sparingly in methyl alcohol, and easily in ethyl alcohol, acetone, acetic ether, and benzol. When recrystallized four times from acetone it formed spindle shaped or rhombic crystals, m p 76° (Pl. I).

Analysis :

Subst. (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)	Mol. wt.
3.471	10.720	2.600	84.23	8.38	
4.170	12.980	3.125	84.90	8.38	
			av. 84.57	8.38	
Calculated for $\text{C}_{31}\text{H}_{36}\text{O}_2$			84.55	8.18	440

Molecular weight (Rast's method):

Subst. (mg)	Camphor (mg)	Depression ($^\circ\text{C}$)	Mol. wt.	
0.300	2.490	11.0	441	
0.333	3.313	10.5	386	av. 414

From the above data, the formula of the crystals is calculated to be $\text{C}_{31}\text{H}_{36}\text{O}_2$.

When 2 g of the crystals were hydrolysed with 8 cc of 10% alcoholic potash, β -naphthoic acid was precipitated as potassium salt. It was filtered off and the filtrate was dissolved in 200 cc of pentane, and after washing with water, the solution was dehydrated with anhydrous Na_2SO_4 and evaporated by a mercury diffusion pump. Pure vitamin A is thus obtained as a red-dish yellow oil.

Analysis :

Subst. (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
3.431	10.485	3.360	83.34	10.96
3.033	9.290	2.950	83.54	10.88
			av. 83.44	10.92
Calculated for $\text{C}_{20}\text{H}_{30}\text{O}$			83.91	10.48

When the potassium salt of β -naphthoic acid above obtained was washed with petroleum ether, and acidified with hydrochloric acid, free β -naphthoic acid separated out, which when recrystallized several times from acetone formed prismatic crystals: m p 183° .

Analysis :

Subst. (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
3.629	10.225	1.575	76.84	4.86
3.074	8.690	1.355	77.10	4.93
			av. 76.97	4.89
Calculated for C ₁₁ H ₈ O ₂ (β -naphthoic acid)			76.74	4.65

The results of the feeding experiment with albino rats are shown in the following charts. (charts I~III).

The author wishes to express his sincere thanks to Prof. U. Suzuki for his kind advices and encouragement throughout this work. He is also indebted to Mr. S. Funahashi for microanalysis.

Appendix

Lately, it has been observed that vitamin A forms also an ester with anthraquinone β -carboxylic acid. When recrystallized from acetone, it forms yellow hexagonal plates: mp 124° (Pl. I). Its activity is estimated to be 6000 C. L. O. units.

Analysis :

Subst. (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
3.944	11.660	2.575	80.63	7.30
3.147	9.283	2.015	80.45	7.16
			av. 80.54	7.23
Calculated for C ₂₀ H ₂₀ O·CO·C ₁₄ H ₇			80.77	6.92

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- (1) Sc. Pap. I, P. C. R., **26**, 77 (1935).
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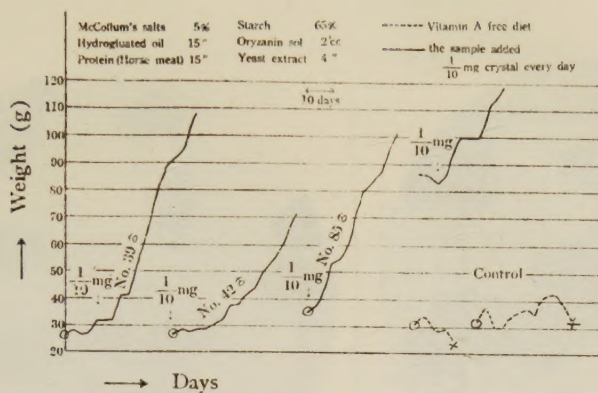


Chart I. Showing the activity of crystalline naphthoic ester of Vitamin A.

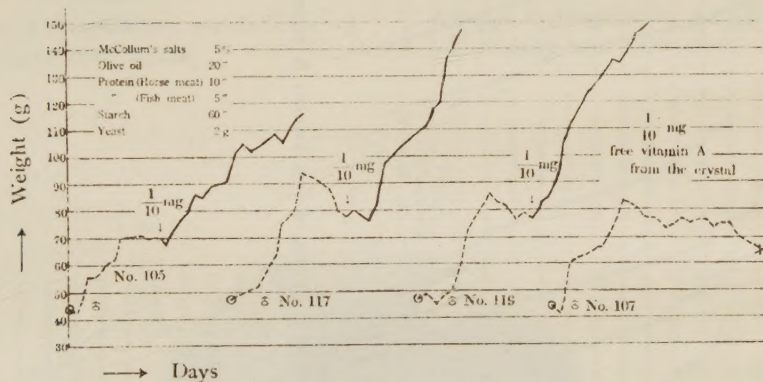


Chart II-(1). Showing the activity of free vitamin A regenerated from naphthoic ester.

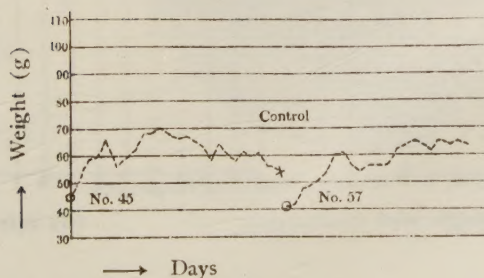


Chart II-(2). Control experiment.

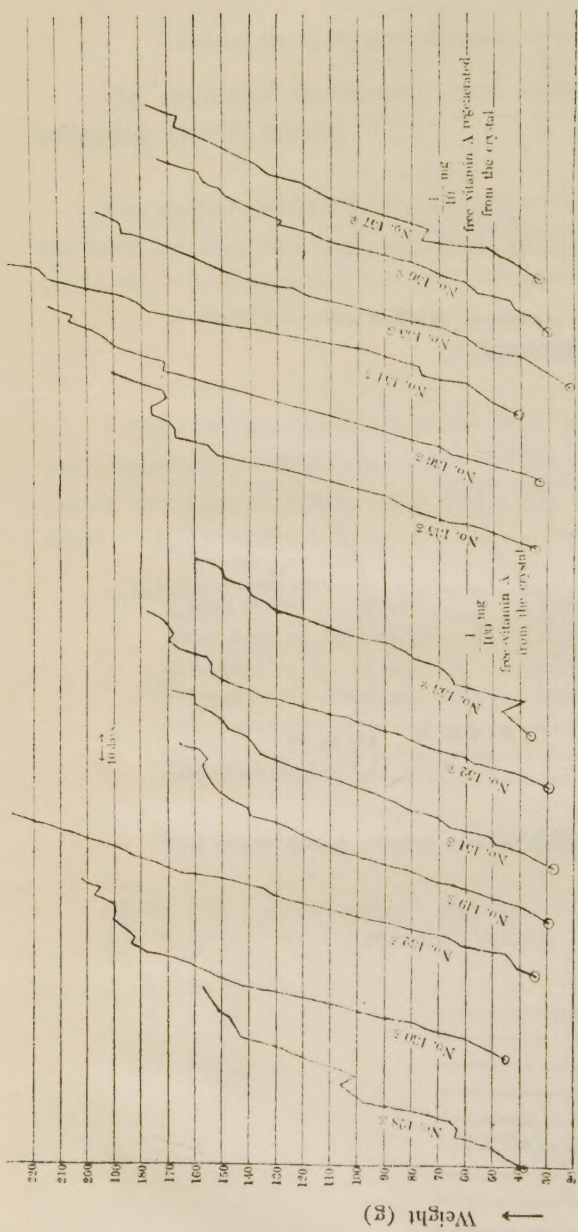


Chart III-(1). Showing the activity of free vitamin A regenerated from naphthoic ester.

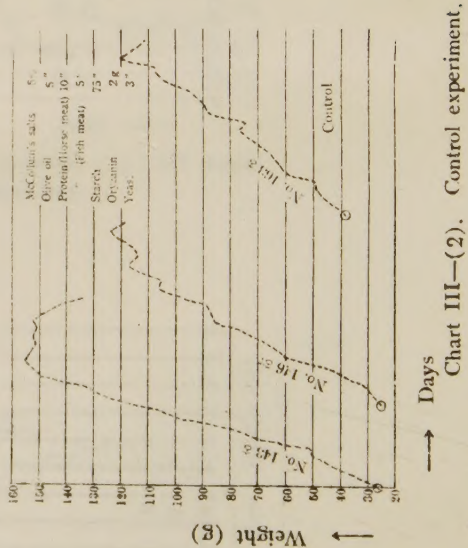


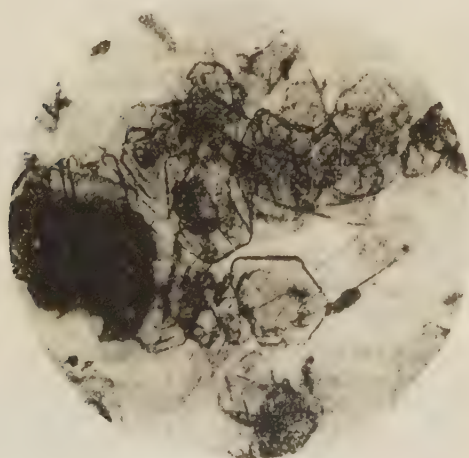
Chart III-(2). Control experiment.



Vitamin A β -naphthoate, m p 76° ,
recrystallized from acetone.



Vitamin A β -naphthoate, m p 76° ,
recrystallized from acetic ether.



Anthraquinone- β -carboxylic ester of vitamin A, m p 124° .
recrystallized from acetone.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Researches on Viscose Pulp and Viscose Pulp Woods. Part VIII.—On the Chemical Composition and Cooking Test of Manchurian Fir (*Abies nephrolepis* Maxim) (pp. 925~930): By Masuzo SHIKATA and Kenkichi TADOKORO.

The chemical composition of Manchurian Fir (*Abies nephrolepis*, Max) is quite similar to Karafuto Fir (*Abies sachaliensis*, Fr. Schm.). As a viscose pulp wood these firs are inferior to Manchurian Spruce (*Picea ajanensis*, Fisch.) and Karafuto Spruce (*Picea ajanensis*, Fisch.).

The cooking test showed, also, that Manchurian fir gives a lower grade pulp than these spruces under the same conditions. However, by the Mg-Ca bisulphite method it gives a comparatively good pulp which can be used as a viscose pulp.

TABLE I. Chemical compositions of Manchurian fir.

	air dried %	oven dried %		air dried %	oven dried %
1 Water	10.90	—	10 Pentosan	12.92	14.50
2 Alcohol-benzene extracts	4.60	5.16	11 Mannan	5.54	6.22
3 1% NaOH soluble matter	12.78	14.34	12 Galactan	0.79	0.89
4 Hot water soluble matter	3.17	3.56	13 Hemicellulose	19.25	21.60
5 Cold water soluble matter	1.12	1.26	14 Methoxyl	5.20	5.84
6 Total cellulose	49.87	55.97	15 Nitrogen	0.05	0.06
7 α -cellulose	27.08	30.39	16 Crude protein	0.34	0.38
8 β , γ -cellulose	22.78	25.58	17 Ash	0.39	0.41
9 Lignin	26.77	30.05	18 Methoxyl/Lignin $\times 100$	—	19.43

TABLE II. Cooking conditions.

Cooking solution	Ca-bisulphite	Mg-bisulphite	Ca + Mg bisulphite
Total SO ₂	6.1	6.1	6.1
Base	CaO, 1%	MgO, 1%	MgO + CaO(1:1), 1%
Maximum pressure	90 lb/sq. inch	90 lb/sq. inch	90 lb/sq. inch
Mean pressure	75 "	75 "	75 "
Maximum temperature	157°C	155°C	155°C
Mean temperature	145°C	145°C	145°C
Time	8 hours	8 hours	8 hours

TABLE III. *Quality of pulps and Viscose threads*

Pulp No.	Kind of Wood	Cooking method	Yields Kg/m ³ wood	Moisture	α -cellu.	Ash	Strength g/denier
1	Shusyo	Ca-sulphite process	166	5.13	80.00	0.71	
2	"	Mg-sulphite process	171	6.24	81.23	0.52	
3	"	Ca, Mg mixed sulphite	172	7.35	84.45	0.44	0.82
4	Gyorinsyo	Ca-sulphite process		6.02	83.18	0.54	
5	"	Mg-sulphite process		9.21	85.76	0.61	
6	"	Ca, Mg mixed sulphite		8.63	86.62	0.41	0.97
7	Ezomatsu	Mg-sulphite process		11.20	85.24	0.38	
8	"	Ca, Mg mixed sulphite process		6.50	86.22	0.41	

Studies on Vitamin C. XVII.—On the Change of Content of Vitamin C in Barley. (pp. 931~942): By Tomiji MATSUOKA. (Ag. Chemical Laboratory, Kyoto Imperial University.)

Die Untersuchungen über Rhizopus-arten (IV). (S. 943~963): Von Yoshito TAKEDA. (Department of Industry, Government Research Institute, Taiwan Japan.)

Chemical Studies on Enzymes of Grain. IV.—On the Distribution of Starchliquefing Enzyme in Rice Grain and the Effects of salts on the Extraction of the Enzyme. (pp. 964~970): By Gohei YAMAGISHI. (Morioka Agricultural College.)

On Saccharomyces Imodane n. sp., Imodane Yeast of Bread. (pp. 971~979): By Satio YAMADA. (Agr. Mycological Laboratory, Tokyo Agricultural College.)

On the Unsaponifiable Matter of the Algae Fats. I.—Sterol. (pp. 980~984): By K. SHIRAHAMA. (Agr. Chemical Laboratory, Hokkaido Imp. University, Japan.)

Having studied on the unsaponifiable matter, the author isolated a certain sterol from *Petelia Wrightii*, Yendo and *Fucus evanescens*, Ag. and this sterol was named "Pervesterol" by the author⁽¹⁾.

Afterwards, Heilbron, Phipers and Wright⁽²⁾ reported in detail on a sterol (Fucosterol) isolated from *Fucus vesiculosus*. Fucosterol might be the same as pervesterol. The results obtained by the author are shown in the following table. On the other hand, pervesterol was found out by the author in *Laminaria longissima*, Miyabe.

The sterols of *Codium fragile*, (Suringer), *Harlot* (green alga) and *Chishima Nori* (red alga) were also separated by the author, but their detailed nature has not fully decided yet. But probably the former might sitosterol.

The sterol of *Chishima Nori* could not be purified because of its yields were too small, but like the other sterol groups, this sterol formed also an alcohol insoluble digitonide. And the Liebermann-Burchard's and Salkowski's tests were shown the same as pervesterol and the reactions with Tortelli-Jaffe's reagent and antimony trichloride in chloroform were negative.

	Sterol		Acetate		Propionate m p	Benzoate m p	Acetate bromide m p
	m p	$[\alpha]_D$	m p	$[\alpha]_D$			
<i>Petelia W.</i>	122°	-39.00	118°	-44.10	104°	114°	133° (decomp.)
<i>Fucus c.</i>	122°	-39.80	118°	-43.2	103°	114°	—
<i>Laminaria l.</i>	123°	—	118°	—	104°	114°	—
<i>Codium f.</i>	130°	—	125°	—	107°	—	—
"Fucosterol"	124°	-38.42	118°~119°	-43.8	105°~106°	120°	133° (decomp.)

(1) K. Shirahama: Reported at 7th Oct. 1933 in the Meeting of the Sapporo Society of Agriculture and Forestry in Japan.

(2) Heilbron, Phipers and Wright: Nature, **133**, 419, (1934); J. Chem. Soc., Oct. 1572 (1934).

Nutritive Value of Canavanin amino acid. (pp. 985~987): By Masayoshi OGAWA. (Department of Nutrition, Tokyo Municipal Hyg. Lab.)

In the previous communications, the author reported that the Canavanin was an essential amino acid for growth, especially in the early life of the animals.

In the present report, the author employed a number albino rats and divided them into two groups, namely Group I and Group II. Fed those two groups with a basal diet which lack of Canavanin. To the first group, gave 0.1 grams of Canavanin per kilograms of the body weight per day.

until their body weight reached to from 100 grams to 150 grams. And then, thereafter discontinued the feeding Canavanin, but continued feeding the basal diet until to their death. On the other hand, to the Group II did not give any Canavanin, but fed with the basal diet alone until to their death.

From the above experiment, the author obtained the following results:

1) The Canavanin seems to be an essential amino acid for growth, especially in the early life of the animals.

2) The animals which received Canavanin in their early life for a certain period grew much better throughout their life than that of the animals which did not receive Canavanin at all.

3) There is not much difference in the prolongation of the life between the Group I and Group II. Therefore, it seems to be that Canavanin has no relationship with the prolongation of the life.

Research on Active Carbon from Karafuto Tundra Peat.

Part. I.—The Comparison of the Raw Materials of Active Carbon. (pp. 988 ~996): By MASUZO SHIKATA and Seiichi NAGANUMA. (Chemical Laboratory for Forest Prod., Kyoto Imperial University.)

In order to find the raw materials of commercial active carbon, several typical saw materials were tested, that is to say, chaffs, bagasse from sugar cane, saw dust, woods (*Quercus serrata*, Thunb. *Quercus glandulifera*, Bl) and tundra peat from Karafuto. Active carbon were prepared under the same—as far as possible—conditions. The methods of activations were chemical activation after carbonizations, simultaneous carbonization with additions of chemicals, activation with super heated steam. It was concluded that the tundra peat is one of the best starting material among the plant products.

On the Optical Properties of the Fermentation Lactic Acids.

Part. I.—The Lactic Acid Bacteria Having the Special Nature of Causing Fermentation Lactic Acids to form Inactive. (pp. 997~1007): By Hideo KATAGIRI and Kakuo KITAHARA. (Agr. Chemical Laboratory, Kyoto Imp. University, Japan)

The form of lactic acid in Sake was ascertained to be inactive, while optically active lactic acid-producing organisms; *Leuconostoc mesenteroides* var. Sake (*l*-former) and the *d*-formers of *Lactobacillus* Sake, were isolated by us [this Bull. 10, 153 (1934)] from yeast mashes for Sake manufacture.

The form of the lactic acid produced by these organisms was found to be very characteristic, since even when the *Leuconostoc* was mixed with any one of the *d*-formers, no modification of the form of lactic acid produced by any of these organisms was observed.

However, the lactic acid produced in the mixed fermentation with *Leuconostoc* and *dl*- or *d+dl*-formers of *Lactobacillus Sake* was found to contain larger amount of racemic form as was calculated.

It was suggested that *dl*- or *d+dl*-formers revealed as *d*-formers when they were taken with the *Leuconostoc* which was superior to the *Lactobacilli*, and when the fermenting power of the *Leuconostoc* was inferior to the *Lactobacilli*, *dl*-formers revealed as *d+dl*-formers producing as much dextro-lactic acid as to cause racemisation of the laevo-lactic acid produced by the *Leuconostoc*.

The inactive-lactic acid producing organism, having very closed characteristics to *Lactobacillus plantarum*, Orla-Jensen, isolated by us from a fermented mash for lactic acid manufacture, was found to reveal very much the same nature as the *dl*-formers above mentioned, when it was mixed with the *Leuconostoc*.

This special nature of *dl*- and *d+dl*-formers was verified when they were inoculated to the Koji-extract added previously dextro- or laevo-lactic acid, the modification of the form of lactic acid produced by them was very remarkable; they produced laevo-acid in the presence of dextro-lactic acid and when laevo-lactic acid was added they revealed as dextro-acid formers, therefore inactive-lactic acid was in each case was obtained.

From these facts, the authors suggested that the reason why inactive-lactic acid would be obtained in natural fermentations, was due, in a great measure, to the special nature of these *dl*- or *d+dl*-formers as was mentioned above.

On the Fermentation Products of Glycerine by the Gluconic acid Bacteria attached to Fruits.—Production of Dioxycetone, Glyceric acid, Acetic acid, Glycollic acid, Succinic acid and a Substance which gives reddish violet color Reaction by Ferric chloride. (pp. 1008~1016): By Teizo TAKAHASHI and Toshinobu ASAI. (Agr. Chemical Laboratory, Tokyo Imperial University, Japan.)
